EFFECT OF AMINO ACID SUPPLEMENTATION AND SOURCE OF PROTEIN IN HIGH-WHEAT MIXTURES ON RESPONSES OF LACTATING DAIRY COWS

By

TOR NALSEN

Bachelor of Science in Agriculture

Oklahoma State University

Stillwater, Oklahoma

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Thesis Approved:

L. J. Bush
Thesis Adviser

David S Buchanan

Donald G Wagner

Norman N. Durham
Dean of the Graduate College

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CHAPTER I

INTRODUCTION

At the present time in Oklahoma there is a surplus of wheat. At times, the price of this cereal grain is low enough that it is competitive with other feed grains used for dairy rations.

The drop in wheat prices will encourage the use of wheat for livestock feed, and the use of it is projected to rise from past seasons. Favorable prices and availability have made wheat an attractive feed grain for milk producers.

For more than 80 years wheat has been fed in place of other grains in dairy rations, but because it has not been extensively used, farmers hesitate to include it in large amounts in dairy rations. The extent to which wheat can be substituted for other feed grains and yet maintain productivity in dairy cows remains to be defined.

In a recent study, Faldet et al. (1986a) substituted wheat for corn in concentrate mixtures fed with sorghum silage as the only forage. Milk yield decreased as the amount of wheat substitution increased, being 28.8, 28.0, and 27.3 kg/day for cows fed mixtures with 0, 40, and 60% wheat. The calculated (NRC, 1985) rumen undegradable protein (RUP) was 8.4% for a concentrate mixture containing corn and 5.8% for a concentrate mixture containing 60% wheat. In that particular trial the amount of lysine expected to escape ruminal degradation was reduced from .41% for the corn mixture to .26% for the mixture containing 60% wheat. In a second trial wherein wheat was 0, 40, 60, or 80% of the concentrate mixture and alfalfa hay was the only forage, milk production tended to decrease also (Faldet et al. 1986b). The amounts of RUP and rumen undergradable lysine also were reduced as the amount of wheat was increased, replacing corn plus soybean meal.

A very important concern of feeding lactating dairy cows is to provide sufficient supply of amino acids to the site of absorption for maximun milk synthesis because of the high demand, especialy in early lactation. Therefore, it is very important to feed high quality protein that reaches the site of absorption. Researchers have suggested that rumen bypass percent and protein quality both must be considered.

There is limited information concerning the site and extent of digestion of wheat by cattle. However, it has been reported that ruminal degradation of wheat is higher than that of other cereal grains (Merterns, 1977; Madsen and Hvelplund, 1985); this could cause duodenal amino acid availability to be deficient in cows on high-wheat rations. Supplementation with protected amino acids could be necesary for availability at the site of absorption. Oltjen et al. (1967) reported that corn is slowly degraded in the rumen. This could be one reason or factor why corn was superior to wheat in some studies. The degradability of the protein is not a positive or negative characteristic of feeds; in some feeding situations, a high and in others a low protein degradability is necessary for optimun production (Madsen and Hvelplund, 1985).

Ruminant animals have two protein requirements, the requirement of the animal itself and the requirement for microbial growth in the forestomach (Tamminga and Van Hellemond, 1977). In young ruminants and lactating dairy cows, only a portion of the protein requirement can be met by microbial protein synthesis. To maximize performance in these stages of production, part of the dietary protein from the basal ingredients or protein supplements must escape rumen degradation and be available for absorption in the small intestine (Chalupa, 1975). He also suggested that to maximize production from ruminants, use must be made of non-protein nitrogen for rumen protein production, maximization of rumen escape protein and supplementation with amino acids that are not degraded in the rumen.

Increased milk production and milk protein have been attained with post-ruminal administration of protein and amino acids (Broderick, 1970; Schwab, 1976) or no effect in cows fed rations supplemented with amino acids (Rogers, 1987).

There is controversy in experimental results as to which is the first limiting amino acid or amino acids for milk production. Methionine has been identified as one of the most limiting amino acid for milk production (Broderick, 1974; Schwab, 1976); lysine may be colimiting (Broderick, 1974) or first limiting in lactating cows (Schwab, 1976).

The objectives of this investigation were to: a) Determine the effects of increasing rumen undegradable lysine and methionine or these plus total escape protein in high-wheat rations with sorghum silage as the only forage. b) Measure the effects of protected amino acids and rumen undegradable protein on certain ruminal and blood metabolites. c) Determine the effects on performance of dairy cows of substituting wheat for corn on a weight basis, without adjusting for protein, using alfalfa hay as the only forage.

CHAPTER II

LITERATURE REVIEW

Wheat is a very important crop in Oklahoma and other Great Plains states. Favorable pricing and availability have made it an attractive feed grain to milk producers; however, its use in livestock rations varies from year to year depending on the availability and price of other feed grains. In some instances, there has been too much reluctance or caution in replacing traditional feed grains with wheat where competition with other cereal grains exists.

Wheat in Concentrate Mixtures

The need for increased efficiency in the dairy cattle industry has stimulated interest in optimizing the feeding value of cereal grains. Researchers through the years have conducted trials on how much wheat would be satisfactory in the ration of dairy cows for maximum production. Most recommendations are to include not more than 30 to 40% wheat in the concentrate mixture.

Feeding trials have been conducted to ascertain the feeding value of wheat (Oltjen et al., 1966; Brethour et al., 1985), but there is limited information concerning the site and extent of its digestion by cattle. The relative value of a cereal grain (Table I) will depend on its proportion in the total diet, type of processing, other dietary constituents and also the level of productivity of the cows (Moran, 1986). Using ruminally canulated animals, Fulton et al. (1979), observed that wheat is a rapidly and extensively digested grain. In general, the physical processing of cereal grains improves their digestion (Toland, 1976). Fulkerson and Michell (1985) concluded that wheat grain should be crushed before fed to milking cows.

The results of trials where wheat was compared with other feed grains are not constant. Tommervick and Waldern (1969) reported no differences in production due to feeding pelleted grain rations containing 96% of either soft white wheat, corn, barley, milo, or oats. Also, there were no differences in milk yield or fat percentage among groups of cows fed concentrates of 98% barley or wheat mixed feed with alfalfa hay as the only roughage (Waldern and Cedeno, 1970). Cunningham et al. (1970), compared two different concentrate mixtures containing soft red winter wheat (33.3 and 67.6%) of two varieties (14 or 18% CP contents) to a corn concentrate mixture with corn silage and alfalfa hay as the forage. Milk production and fat percentage were lower ($P<.05$) for the cows where wheat comprised 66.7% of the concentrate than for cows fed a concentrate mixture with 33.3% wheat and cows on the high protein wheat lost weight. They suggested that development of varieties of wheat with

TABLE I

COMPARATIVE NUTRITIVE VALUE OF GRAINS[a]

Item	Corn	Barley	Sorghum	Oats	Wheat
	(% or Mcal/kg, as fed basis)				
Dry matter	89.0	88.0	90.0	89.0	89.0
Crude protein	9.6	11.9	11.1	11.8	14.2
Net energy	1.78	1.71	1.78	1.57	1.81
Crude fiber	2.6	5.0	2.4	10.8	2.6
Ash	1.3	2.3	1.8	3.1	1.7
Calcium	.03	.04	.03	.07	.04
Phosphorus	.26	.34	.29	.33	.37
Amino acids					
Lysine	.25	.39	.25	.39	.37
Isoleucine	.35	.45	.45	.43	.47
Leucine	1.21	.75	1.44	.81	.87
Methionine	.17	.15	.13	.17	.18
Phenylalanine	.48	.58	.56	.52	.61
Threonine	.35	.37	.36	.36	.38
Valine	.44	.57	.52	.56	.57
Tryptophan	.08	.15	.11	.15	.15
Arginine	.43	.51	.39	.70	.59
Histidine	.26	.24	.23	.18	.29

[a]United States - Canadian Tables of Feed Composition, 1982 (Third Revision).

high yielding potential and higher protein content would result in more economical dairy rations.

McPherson and Waldern (1969) reported that production of 4% fat corrected milk and milk composition were unaffected due to feeding pelleted concentrate mixtures containing from 20 to 93% steam-rolled soft white wheat, with alfalfa hay as the only roughage. There were no off-feed or digestive disturbances.

Criberio et al. (1979) noted no significant differences for 4% fat-corrected milk (averaging 12.4 kg/day), milk protein, total solids and non-fat solids content when cows were given concentrate mixes where wheat was none, 19, 38, 57 or 77% of the mix replacing mainly corn. The cows had a significantly lower dry matter and metabolizable energy intake on the mixtures where wheat replaced 57 and 77% of the corn. Moran (1983), reported that cows fed 60% rolled barley produced less milk (22.9 kg/day) than those fed wheat or oats (24.0 and 25.1 kg/day). Milk protein was higher for cows fed wheat than for those fed barley or oat mixes, being .89, .80 and .78 kg/d, respectively, and reflected dietary crude protein content of the diets (18, 16.9, 17.0%, respectively).

Moran (1986), fed complete rations to 18 crossbred Friesian cows. Three in their first and 15 in their second or later lactations were divided into three groups on the basis of stage of lactation (69 days postpartum), milk yield and live weight. Diets contained 60% of wheat, barley or oats, 17.5% oaten silage, 17.0% lucerne hay, 3.0% meat and bone meal, 1.0% urea and 1.5% mineral and buffers. Dry matter intake was not affected by the diets averaging 17.6 kg/day. Cows on the oat diet produced more fat-corrected milk than those fed wheat or barley (27.6 vs. 24.9 and 24.6 kg/day, respectively). Cows on the wheat ration produced more milk protein concentration than those fed barley or oats (38.4 vs. 35.2 and 31.2 g/kg, respectively).

Faldet et al. (1986a) fed cows concentrate mixtures where wheat replaced corn. The mixtures were fed in a complete ration using sorghum silage as the only forage. There was a linear decrease ($P<.001$) in milk yield as the amount of wheat in the ration increased, being 28.8, 28.0 and 27.3 kg/day for cows fed mixtures with 0, 40, and 60% wheat. The calculated amount of RUP and particularly lysine and methionine was reduced when wheat replaced corn and some soybean meal. In a second trial (Faldet et al., 1986b), milk production tended to decrease also as the amount of wheat increased (0, 40, 60 or 80%) in the concentrate mixture and alfalfa hay was the only forage. The amounts of RUP and rumen undergradable lysine also were reduced as the amount of wheat was increased.

Wheat has been compared with other cereal grains and it seems to be reasonable to conclude that it can be incorporated safely into the diet in lactating dairy animals. The differences in responses in various trials could be attributed to the different types of forage used, lactation stage, types of wheat, type of grain replaced by wheat, method of processing, or some combination of these factors.

Responses of Cows to Protein Intake

Finding the optimum protein content of the feed (from an economic point of view) requires a knowledge of the quantitative relationship between protein level, milk yield, live weight gain and different phases of the lactational period (Danfaer et al., 1980). Milk production can be affected by protein intake by providing more amino acids, increasing available energy and by altering the efficiency or pattern of absorbed nutrients (Chalupa, 1984).

In ruminant animals, the amount and composition of protein digested and absorbed in the small intestine may be very different in terms of both amount and composition from that in the diet because considerable destruction of dietary protein occurs in the rumen (Orskov, 1970). The requirement for protein absorbed from the small intestine is mainly governed by the level of production and will be relatively high for milk production, particularly in early lactation (Tamminga, 1979), and it is likely that the protein content of the diet limits milk yield in this period (Danfaer et al. 1980).

Considerable controversy exists concerning the optimum concentration of crude protein in diets for lactating cows. NRC (1978) recommends that diets for lactating cows contain

13 to 16% CP depending on the stage of production and intake of the cows.

More efficient utilization of dietary protein in dairy rations is needed because of increasing cost of protein supplementation. Majdoub et al. (1978) fed cows in their 8th to 10th week of lactation rations having two different percentages of protein (13 and 15%) and two nitrogen solubilities (22 and 42%). The concentrate mixtures were fed in complete rations (60% concentrate + 40% sorghum silage on a dry basis). Wheat middlings and wheat bran were used in the high solubility rations. Solubility of nitrogen did not affect average daily intakes of dry matter, crude protein, or net energy for lactation. However, average daily intakes of these were increased in cows fed rations with the higher percentage of protein. The lower nitrogen solubility resulted in increased milk yield. No significant effects were detected in percentages of total solids, fat, protein, and non-fat solids of milk. Gordon and McMurray (1979) fed cows in their first lactation during the first 75 days post-calving grass silage ad libitum supplemented with 8 kg. of concentrate per day. The treatments consisted of six supplements designed to contain 9, 13, 17, 21, 25 and 29% crude protein on a fresh basis. All the supplements contained equal proportions of ground maize, molasses and minerals, but ground barley was progressively replaced by extracted soybean meal to increase the protein content of the concentrate. The response of milk yield during the final week of the experiment was quadratic ($P<.05$); maximum milk yield was achieved when the supplement contained 24.4% protein. There was a significant quadratic ($P<.08$) response in the protein intake. The maximum milk protein percentage was attained when the supplement contained 23.9% protein. Protein content of the supplement had a highly significant effect on the urea-N content of blood serum with the urea concentration increasing linearly with increasing protein intake. McLeod et al. (1982) also noted a curvilinear increase in milk yield and linear increases of feed intake and digestibility when Holstein cows in first lactation were fed diets that contained 12, 15, or 18% crude protein.

Danfaer et al. (1980) reported that fat-corrected milk yield in cows fell from 24.5 to 23.1 kg/day as diet crude protein increased from 19 to 23%. Greater differences were attained between 11 and 14% than between 14 and 17% CP in a study done by Kung and Huber (1983) where cows were fed three different amounts of protein (11.3, 14.5 and 17.5%). Also, similar results were reported by Chalupa (1984). In this review of several studies where corn and soybean meal were the based diet, larger increases of milk yield were obtained when crude protein concentrations were raised from 9 to 10% than when raised from 13 to 14% and increasing crude protein above 14% resulted in a smaller and declining rate of increase.

Van Horn (1982) reviewed several studies where soybean meal was used as reference standard to increase CP in the ration because of high quality and widespread use. Responses were noted when soybean meal was added to rations to increase the CP above 11% but responses were more variable when soybean meal was added to ration to increase the CP above 12% and that milk yield response diminished above 14% CP in the ration. To select the optimum protein level an economic decision must be made. Increasing the protein concentration in the diet beyond a certain point results in a decrease in milk production. However, the response differences in feeding different amounts of protein may be due to the source of protein, stage of lactation, parity of cows and amount fed.

Rumen Undegradable Protein

In feeding ruminant animals, one has to consider two protein requirements, the requirement of the animal itself and the requirement of the microbial population in the forestomach. Meeting the requirement of the animal means supplying adequate amounts of essential amino acids and sufficient nitrogen, carbon (C) and energy for synthesis of necessary non-essential amino acids (Tamminga and Van Hellemond, 1977). These components may come from either undegradable protein or degradable protein which is transformed into microbial protein (Chalupa, 1975). He suggested that degradable protein in excess of rumen microbial requirements is wasted, and provided there is sufficient degradable protein to meet microbial requirements, increasing dietary undegradable protein will increase flow of protein to the intestine.

Because of the inadequate support of microbial protein for milk production, much interest has been focused in the amount of protein that escapes degradation in the rumen. As production levels increase, the portion of protein that escapes or is not degraded in the rumen must increase (NCR, 1985). Even though considerable destruction of dietary protein occurs in the rumen, increase of protein that is needed to reach the small intestine could be either by increasing dietary protein (Orskov, 1970; Chalupa, 1975) or by reducing degradation of the protein already present in the diet (Chalupa, 1982).

It has been suggested that rumen microorganisms appear to provide sufficient amino acids to the tissue system for maintenance, slow growth and early pregnancy but not for fast growth, late pregnancy or early lactation (Orskov, 1970); therefore, increasing the amount of dietary undegradable protein reaching the small intestine is essential during early lactation (Chalupa, 1982).

Definitive data on protein degradability is lacking. The variation in degradation of dietary protein in different experiments must be attributed to techniques used to measure, nature and solubility of dietary protein, rate of passage and level of feed intake (Tamminga, 1979). There is also a difficulty in distinguishing feed proteins from microbial protein and endogenous secretions. Degradation also varies with changes in rumen environment (Chalupa, 1982), class of animal, production level and among animals within classes (Barrio et al., 1986).

There can be wide differences among feed ingredients in the extent of ruminal degradation. Results of several studies indicate that as little as 40% or as much as 80% of dietary protein normally might be degraded in the rumen and transfered into microbial protein (Chalupa, 1975). The protein of small grains, such as barley, oats and wheat is more degradable than that of corn and milo (Mertens, 1977; Chalupa, 1982). Lebzien et al. (1984) fed rations where wheat and maize protein provided 39.5 and 28.9% of the total crude protein. The proportion of rumen undegraded feed protein was 25% with maize and 9% with wheat. Khadanovich et al. (1986) fed mixtures containing 0 and 29% wheat to lactating cows. Average daily milk yield was 22.06 and 19.56 kg/day and degradability of the protein of the feed mixtures in rumen liquid was 77.0 and 87.4%.

There is considerable interest in protein sources that are slowly degraded in the rumen. Barrio et al. (1986) reported that disappearance of protein in the rumen after 12 hours of incubation in nylon bags was 89.7% for wheat and 41.6% for corn in crossbred steers fed a 40% concentrate ration consisting of corn and soybean meal fed with prairie hay as the forage component. Loerch et al. (1983) reported no differences between soybean meal and blood meal in nitrogen digestibility by sheep, but in steers supplemental protein escape was 28.7 vs. 81.7% for soybean meal and blood meal, respectively.

There are various ways to decrease the extent of degradation of dietary protein in the rumen. One way is by using heat and chemical treatments; another method includes formulation of diets from ingredients with low protein solubility with natural resistance to ruminal breakdown. These relatively resistant protein sources can have special value for lactating cows in early lactation and for growing ruminants whose protein requirements are relatively high (Tamminga, 1979). Formulating diets on basis of protein solubility has improved protein utilization in lactating dairy cows (Majoub et al., 1978). It is important that the procedures used to reduce the extent of degradation in the rumen do not interfere with ruminal metabolism or post-ruminal digestion.

It has been suggested that unless milk production exceeds 25 kg/day, which is often the case in early lactation, under most feeding conditions protein supply in the small intestine is sufficient (Chalupa, 1975). Majoub et al. (1978) suggested that ingredients used to increase dietary crude protein above 12 to 14% should contain proteins which are resistant to ruminal degradation because at this level of crude protein there is probably a ratio of degradable protein to energy in the rumen such that the major portion of the protein degraded will be reformed into microbial protein. At higher levels of dietary protein additional degradable protein will be wasted because there will not be enough energy to reform degraded protein into microbial protein.

There may be a need to investigate the possibility of including byproduct feeds (i.e., corn gluten meal, blood meal, etc.) which contain proteins that are less degraded in the rumen than cereal grains and oilseed meals (Chalupa, 1982) in concentrate mixtures containing a high percentage of wheat to feed lactating dairy cows.

Limiting Amino Acids for Milk Production

All animals have a nutritional need for essential amino acids (EAA) and non-essential amino acids (NEAA). Proteins are required to furnish the animal with amino acids which are necessary for various essential synthetic processes in the body. The problem with ruminants in relation to protein amino acid nutrition is the correlation of dietary amino acid concentration in the diet with the amino acid supply reaching the small intestine (Bergen, 1986). The amino acid pattern entering the small intestine is strongly influenced by microbial protein (Santos et al. 1982). The microbial population in the forestomach of the ruminant has the capacity to synthesize all essential amino acids and non-essential amino acids (McDonald et al. 1981). Therefore, it has been suggested that protein quality is not as important to a cow as it is for non-ruminant animals. Virtanen (1966) fed cows purified diets in which urea and ammonium salts accounted for 95.5% of the total nitrogen. Cows not only survived and maintained themselves but produced as much as 10,000 lbs of milk in a year and reproduced normally. This experiment demonstrated that the microflora of the rumen are able to synthesize all of the amino acids and that the amino acids formed are used for the synthesis of milk protein. The major contributions to intestinal amino acid supply in ruminants are amino acids from microbial protein and those from feeds which have escaped ruminal degradation. It has been suggested that the major source of amino acids reaching the absorption sites in ruminants come from microbial protein synthesized in the rumen (Clark, 1975), and that 60 to 80% of the amino acids absorbed from the intestine come from microorganisms under most conditions (Tamminga and Oldham, 1980). Some results on the apparent absorption of the total amino acids indicate that 65 to 75% of those entering the small intestine are apparently absorbed (Tamminga and Van Hellemond, 1977; Santos et al., 1982).

The tissue requirement for amino acids of a dairy cow consists of essentially two elements, maintenance requirement and a requirement for milk synthesis (Tamminga and Van Hellmond, 1977). Clark (1975), recommended that protein nutrition in ruminants must be evaluated in terms of the amino acids that are absorbed from the gut in relation to these two requirements and that even with diets containing recommended quantities of protein, the low supply of amino acids reaching absorptive sites in the lower gut may limit milk production. Also, because of the lack of some key nutrient many cows may fail to produce to their genetic capacity, especially if production potential is in excess of 30 kg. of milk daily.

The mammary gland in dairy cows is the major site of amino acid utilization for synthesis of milk (Tamminga and Oldham, 1980; Nephan, 1982), and the total amino acid requirement depends to a large degree on the requirements for milk production (Tamminga and Van Hellemond, 1977). This high demand of the mammary gland to produce high milk yield could decrease if there is a shortage of a specific nutrient. This shortage could be the result of limited nutrient intake because of limited appetite or physical capacity of the animal or the inability of the rumen microorganisms or tissues of the animal or both, to produce sufficient quantities of precursors to meet this high demand (Clark, 1975).

The requirement for an amino acid(s) is the minimum amount needed to establish a particular level of production (Oldham, 1980). One method for identifying essential amino acids is based on the hypothesis that an essential amino acid(s) concentration will increase in blood plasma only when supply is in excess of requirement (Broderich, 1974; Clark, 1975). This concentration will reflect a balance between intestinal absorption plus endogenous synthesis and utilization of amino acids (Clark, 1975).

The efficiency with which amino acids are extracted from the arterial blood to the mammary gland has also been used to predict limiting amino acids. One criterion is that those amino acids which are in shortest supply relative to demand will undergo minimal metabolism within the gland and a second criterion is that they will be extracted most efficiently from arterial blood supply to the gland. On this basis, methionine, phenylalanine, leucine and threonine are the most limiting amino acids (Oldham, 1980).

Methionine has been suggested as one of the first limiting amino acid for milk protein synthesis (Chandler and Polan, 1972; Chalupa, 1975; Clark, 1975). Particularly in high-yielding dairy cows, the demand for methionine is expected to exceed the available supply. In in vitro studies, methionine has been found to stimulate microbial growth, increase cellulose digestion and increase microbial fatty acid synthesis (Oldham, 1980).

There is evidence that no single amino acid is clearly limiting for milk production (Tamminga and Van Hellmond, 1977). In attempts to increase the amino acid supply for milk production, post-ruminal infusions of casein or of mixtures of essential amino acids were more successful than infusions of individual amino acid (Clark, 1975). This would indicate that no single amino acid is the limiting one.

Chandler and Poland (1972), calculated minimum transfer efficiencies from blood to milk protein for serum essential amino acids and suggested that methionine, lysine, phenylalanine, tyrosine, and threonine were the most limiting. Broderick et al. (1974) reported that lysine, methionine and valine were most limiting when incremental amounts of formaldehyde treated casein were added to a basal ration of 9.0% crude protein to achieve rations containing 11.2, 13.5, 15.7, and 18.0%.

Lewis and Emery (1962) fed mature cows fitted with ruminal fistulas 6 to 8 lb. of alfalfa hay and 10 to 14 lb. of a 16% protein concentrate mixture and indicated that arginine and threonine were degraded rapidly; lysine, phenylalanine, leucine, and isoleucine were intermediate, and valine and methionine were the amino acids least rapidly degraded.

Most of these studies have been done on cows in different stages of production, age, environment and with different types of feed, feed intake, and degradability. Because of these factors the amount and ratio required by the animal for specific amino acids may be different for milk production.

Amino Acid Supplementation of Dairy Concentrate Mixtures

In feeding high-yielding dairy cows it is critical to provide an adequate amount of amino acids to the site of absorption, particularly in early lactation when the cow is mobilizing body tissue to compensate for inadequate nutrient intake. One way to insure that adequate amino acids reach the lower gut is to increase protein that would resist ruminal degradation or by supplementing rations with rumen protected amino acids, or both. By adding those amino acids which are expected to limit milk production to rations that have insufficient amounts, one might expect to increase production. The manipulation of amino acids is important because we would like to maximize lactation performances with minimum wastage of dietary protein.

Casein has been used extensively to demonstrate the benefits of providing more protein to the small intestine for digestion and absorption, thus increasing the postruminal supply of amino acids. The reported increase in milk yield and increase in milk protein production by dairy cows postruminally infused with casein (Clark, 1975) have demonstrated that individual amino acid(s) are potentially limiting the production of milk giving rise to interest in making rumen protected forms of amino acids. Broderick et al. (1970) obtained significant increases in milk protein and milk production when methionine supplemented casein was infused abomasually into cows fed a 16% CP diet. Other studies with postruminal infusion of essential amino acids, either singly or in combination, have not given consistent positive responses (Schwab and Satter 1973, 1974).

Lysine or methionine, or both, have been identified to be the most limiting amino acids for milk production or milk protein (Broderick et al. 1974; Schwab et al. 1976). Because of such findings, analogs, derivatives, and encapsulated methionine or lysine, or both, have been made to supplement dairy rations. Processing the amino acids to make the supplements should not interfere with the absorption and utilization of it (Tamminga and Van Hellemond, 1977). Holter et al. (1972) and Polan et al. (1970) have observed an increase in milk fat production due to supplementing rations with methionine hydroxy analog and Griel et al. (1968) observed increased production in fat-corrected milk. On the contrary, Broderick et al. (1970) reported that feeding 5, 15, or 45 g/day of encapsulated methionine to lactating dairy cows on a corn based ration containing 15 to 16% crude protein had no effect on milk production or composition.

Huber et al. (1984) reported that in cows fed a 16% CP ration supplemented with 25 g/day of methionine hydroxy analog, milk yield was not affected but fat percent and fat yields were increased 21 and 17% by the additive. Feeding corn-based rations with a crude protein content of 15.8%, Casper et al. (1987) reported that supplementation with methionine to cows did not affect milk production or milk fat percentages but it did increase milk protein percentage. Illg et al. (1987) fed cows diets of corn and SBM and reported a 2% relative increase in the percentages of milk protein and approximately 7% increase in milk production by providing a supplement of 15 g/day of ruminally protected methionine.

Schwab et al. (1976) reported that abomasal infusion of methionine had no effect on milk yield, milk protein or milk fat. Infusion with lysine resulted in 16% of the total response in milk protein production that was obtained with either the 10 essential amino acids or sodium caseinate. An infusion of lysine and methionine together accounted for 43% of the total response. They suggested that lysine and methionine are first and second limiting or co-limiting for production of milk protein in cows fed corn silage, limited alfalfa hay and a concentrate containing 61% corn. Oldham (1980) suggested that in this particular trial lysine was probably first limiting because the ration used was largely based on corn products which likely reduced abomasal lysine proportions. Rogers et al. (1987) also reported an increase in production of milk protein using rumen-protected methionine and lysine. They suggested that lysine appeared to improve the utilization of methionine and reported no differences on feed intake, milk yield and 4% fat-corrected milk with supplementation with rumen-protected lysine. There was a linear increase of plasma methionine and lysine as the amounts of these amino acids supplied postrumminally increased.

The decrease in milk production observed in some studies where cows are fed large quantities of wheat could be because wheat is degraded more extensively in the rumen than are other cereal gains (Mertens, 1977; Madsen and Hvelplund, 1985). Because of this, the amino acid supply to the lower gut may be unbalanced. Even though the levels of lysine and methionine are higher in microbial protein than in feed ingredients commonly fed to ruminants (Santos et al. 1982), supplementation with rumen protected lysine and methionine to these wheat rations may help supply the large demand for these two essential amino acids for milk production.

CHAPTER III

AMINO ACID SUPPLEMENTATION OF HIGH-WHEAT CONCENTRATE MIXTURES FOR DAIRY COWS

Summary

The effect on milk yield of protected amino acids (lysine and methionine) or high rumen escape protein was determined using 24 Holstein cows in their second or later lactation. Treatment were: (a) Positive control (with corn at 45 and barley at 10% of the concentrate mixture), (b) Negative control (wheat at 60% having low rumen undegradable protein), (c) Diet "b" plus supplemental protected amino acids, and (d) High wheat with RUP equal to the positive control plus supplemental amino acids. These concentrate mixtures were fed in complete rations with sorghum silage as the only forage. Total dry matter consumption was similar for all treatment groups. Cows fed high-wheat rations, with or without supplementation with protected amino acids, produced less milk than cows fed the control ration (29.1 vs. 30.8 kg/day). Increasing the rumen escape protein content of the high-wheat ration by substituting corn gluten meal and blood meal for soybean meal plus adding protected amino acids restored milk yield to that of cows fed the control ration. Milk fat test was greater for the wheat

groups than that of the control group, and milk protein was slightly greater for the cows fed the wheat rations supplemented with protected amino acids than for cows fed the other rations. Supplementation with amino acids did not affect body weight, condition score changes, or rumen pH 4 hours after feeding. Rumen pH at 2 hours was lower for cows fed all the wheat rations than for those fed the control ration. Rumen ammonia-N was lowest at 2 and 4 hours in cows fed the wheat ration high in RUP. Blood plasma lysine concentration was higher for cows fed wheat ration low in RUP supplemented with amino acids than for cows on the other wheat rations.

Introduction

In recent years, the availability of surplus wheat has made it an attractive feed grain for dairy rations. The use of wheat in dairy rations has been affected by the traditional use for human consumption and the reluctance or caution about how much wheat can be substituted for traditional feed grains.

Faldet et al. (1986a) substituted wheat for corn in concentrate mixtures containing sorghum silage as the only forage. Milk yield decreased as the amount of wheat substitution increased, being 28.8, 28.0, and 27.3 kg/day for cows fed mixtures with 0, 40 and 60% wheat. The rumen undegradable protein (RUP) was calculated (NCR, 1985) to be 8.4% for the concentrate mixture containing corn and 5.8%

for the mixture with 60% wheat. In particular, the amount of lysine in the concentrate mixture that would be expected to escape ruminal degradation was reduced from .41% for the corn mixture to .26% for the mixture containing 60% wheat. In a second trial wherein wheat was 0, 40, 60 or 80% of the concentrate mixture and alfalfa hay was the only forage, milk production also tended to decrease (Faldet et al. 1986b). The amounts of RUP and lysine escape were reduced in the rations in which wheat was substituted for corn plus soybean meal.

Cunningham et al. (1970) compared two varieties of ground soft red winter wheat (14 or 18% CP contents) partially replacing corn and soybean meal at a level of 33.3 or 66.7% of the concentrate mixture. Milk production and fat percentage were lower ($P<.05$) where wheat comprised 66.7% of the concentrate than when it comprised 33.3% of the mixture.

This experiment was conducted to determine the effects of increasing rumen escape lysine and methionine or these plus total escape protein in high-wheat concentrate rations on the performance of dairy cows.

Materials and Methods

Twenty-four Holstein cows in their second or later lactation were used in a feeding trail starting 6 to 8 weeks postpartum. A switchback design with three four-week periods was used (Lucas, 1956). The first two weeks of each period were used for adjustment to the rations and recovery from any carry-over effects, whereas the last two weeks were used for comparisons among treatments. The cows were randomly assigned to a treatment sequence. Twelve treatment sequences were used, each of which included two treatments (Appendix, Table XV). One treatment was applied in the first and third periods, and the other applied during the second period. All treatments were applied the same number of times. Four treatments were included in the experiment: (a) Positive control (with corn at 45 and barley at 10% of the concentrate mix), (b) Negative control (wheat at 60% of the concentrate), (c) Diet "b" plus supplemental protected lysine and methionine[3]; and, (d) High wheat mixture with RUP calculated to be equal to the positive control by substituting corn gluten meal and blood meal for part of the soybean meal plus the addition of protected amino acids. The mixtures were by calculation nearly equal in net energy and total protein content (Table II). The concentrate mixes were included in complete rations with sorghum silage as the only forage at a rate of 55% concentrate to 45% forage on a dry matter basis.

Cows were fed equal portions of the rations in individual stanchions three times daily at 8 hour intervals

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[3]Product supplied by the Eastman Kodak Co; composed of polymer coated cores which supply 36.4% protected L-lysine and 11.6% protected DL-methionine to the small intestine.

TABLE II

CONCENTRATE MIXTURES FED WITH SORGHUM SILAGE[1]

Composition	RATION Control	Low (RUP)	Wheat (Low RUP+AA)	Wheat (High RUP+AA)
Ingred., (% as fed)				
Wheat[2]		60	60	60
Corn	45	--	--	6.6
Barley	10	--	--	--
Oats	5	5.5	5.5	5
Corn gluten meal	--	--	--	9
Blood meal	--	--	--	2
Soybean meal	25	20	20	3
Cottonseed meal	5	4.5	4.1	4
Fixed portion[3]	10	10	10	10
Protected lysine and methionine[4]	--	--	.4	.4
Calculated analysis (as fed)				
Net energy Mcal/100 kg	158.6	160.6	159.9	161.5
Crude fiber, %	6.3	6.2	6.2	5.5
Total protein, %	19.0	19.0	19.1	19.0
Rumen undeg. protein, %	7.56	5.41	5.55	7.63
Rumen undeg. lysine, %	.36	.24	.38	.38

[1]Concentrate: forage, 55:45 (dry basis); total ration (dry): NE_1 157.5 Mcal/100 kg., total protein 14.5%, crude fiber 16.8%.

[2]Hard red winter wheat, No.2 grade, test wt. 60 lb/bu.

[3]Fixed portion of concentrate mix: Cottonseed hulls 5, limestone 1.5, dicalcium phosphate 1.25, sodium bicarbonate 1.0, salt .75 and magnesium oxide .5%.

[4]Product of Eastman Chemicals Division, Eastman Kodak Co., Rochester, NY; 37% lysine and 11.8% methionine content.

(1100, 1900 and 0300 hours). Enough feed was offered to the cows to allow a minimum amount of feed refusal. The adjustment of the feed was accomplished by increasing or decreasing the amount of concentrate by .45 kg. (as fed basis) and the corresponding amount of sorghum silage by the appropriate amount to maintain a constant ratio of concentrate to forage.

Feed intake was recorded daily and feed weighbacks for each cow composited on a weekly basis for determination of dry matter (DM) by drying in a forced air oven at 60C for 48 hours and for crude protein (N x 6.25) by the macro-kjeldahl method (A.O.A.C., 1975). The cows were milked twice daily (0500 and 1500 hours), milk weights were recorded daily and samples were obtained at four consecutive milkings each week for fat percentage using a Milko Tester MK III F-3140 and protein percentage using a Pro-milk MK II F-12500. Cows were weighed on two consecutive days at the beginning of the trial and at the end of each period. Because the cows were weighed before the p.m. milking, the subsequent milk weight of each cow was subtracted from the respective body weight.

Body condition of each cow was evaluated using the scoring system described by Aalseth et al. (1983) at the beginning of the trial and at the end of each period.

On the last day of each period, at 2 and 4 hours after the 11:00 a.m. feeding, a sample of rumen fluid was taken by stomach tube. A minimum of 350 ml of rumen fluid was collected, strained through a double layer of cheesecloth;

pH was measured immediately with a pH meter. Then, 200 ml of the strained rumen fluid was acidified with 8 ml of 50% hydrochloric acid. This was done to stop microbial activity and to bind the ammonia as ammonium chloride. Samples were frozen in order to preserve them for later analysis. Later, samples were thawed and centrifuged (1000 x g) for 10 minutes and two 50 ul aliquots of the supernatant solution were assayed for rumen ammonia nitrogen (NH_3-N) following the procedure of Broderick and Kang (1980). Using a Varian DMS 90 spectrophotometer at a wavelength of 630 nm, the concentration of ammonia was determined.

One hundred ml of the strained rumen fluid was mixed with 1 ml of a saturated solution of mercuric chloride and then frozen for later volatile fatty acid (VFA) determination. Samples were thawed and centrifuged 10 minutes at 2000 x g. Then, one ml of 20% meta-phosphoric acid was added to 5 ml of the supernatant solution and centrifuged for 20 minutes at 25,000 x g. The meta-phosphoric acid was used to convert the VFA that are in a salt form to a free acid form in order to determine VFA concentration by gas-liquid chromatography. One ml of supernatant solution was combined with .2 ml of 2-ethylbutyric acid as an internal standard and samples were then subjected to gas-liquid chromatography for VFA analysis. The internal standard was made by diluting .5 ml of 2-ethylbutyric acid to a volume of 100 ml with water.

At the same time that the rumen fluid was taken, two samples of blood were taken from the median caudal vein. One sample was withdrawn into a 15 ml vacutainer tube and 1.5 ml of oxalic acid was added and mixed to prevent coagulation. The samples were immediately chilled in ice-water and later centrifuged at 2000 x g for 30 minutes. Plasma was separated and frozen for later analysis of plasma urea nitrogen concentration as described by Fawcett et al. (1960). The second blood sample was withdrawn into a 15 ml heparinized vacutainer tube and placed in ice water. Samples were centrifuged at 3000 x g for 15 minutes, then 2 ml of plasma was mixed with .20 ml of deproteinizing solution in a centrifuge tube, shaked well and centrifuged at 4000 x g for 15 minutes. The deproteinizing solution consisted of 25 g of sulfosalicyclic acid mixed with distilled water and diluted to 50 ml volume. One ml of clear deproteinized plasma was withdrawn and stored at -80C for later analysis of plasma amino acid concentration by the Eastman Kodak Co. Research Laboratories.

For estimating digestibility of different nutrient components of the ration, chromic oxide (Cr_2O_3) was included in the concentrate mixture at a level of .28%. It was used as an external marker to calculate DM and CP digestibility for each ration. Cows were fed the chromic oxide the last 21 days of the second period and fecal grab samples were taken from all 24 cows on the last 5 days at 0400 and 1600 hours. Corrections for diurnal variation were made on the basis of data obtained by collecting feces from 12 cows every 4 hours for 2 days. To determine total chromium intake, samples of the grain and refusals were analyzed for chromium concentration. Chromium in the samples was estimated by using a Varian DMS 90 spectrophotometer at a wavelength of 400 nm. (Appendix, Table XXV).

Data for the different response variables were summarized on a "per period" basis for further statistical analysis. An analysis of variance (Lucas, 1956) was performed on the data collected on the trial for the different response variables using the Statistical Analysis System (SAS). The adjusted treatment means were compared using a pre-planned contrasts as follows: Control mixture vs. wheat mixture calculated to be low in RUP; wheat mixture low in RUP vs. wheat mixture low in RUP supplemented with amino acids; and, wheat mixture low in RUP vs. wheat mixture high in RUP.

Results and Discussion

Intake of DM from concentrate was similar for all treatment groups averaging 14.80 kg/day (Table III). Intake of wheat by cows fed mixtures containing wheat averaged 8.9 kg/day (dry basis). Also, total DM intakes were similar for all treatment groups averaging 26.75 kg/day. No problem with off-feed or digestive disturbances occurred as a result of this amount of wheat. This was in agreement with observations by Faldet et al. (1986) where the mixture fed contained up to 60% wheat and sorghum silage was the only forage. Neither addition of protected amino acids to the ration nor protected amino acids plus an increase in RUP had any influence on feed intake.

Intake of total protein exceeded NRC requirements (NRC, 1978), from 25 to 32.3% (Table III). This was due to the calculated values for milk production that were for cows expected to milk on an average of 36.40 kg/day, whereas actual production ranged from 29.03 to 30.82 kg/day. By the formula of Conrad, predicted DM intake for cows producing 28.5 kg/day of 4% FCM would be 21.7 kg/day, or about 3.4% of the body weight. In this trial, the average intake was 4.2% of the body weight. Crude protein intakes as a percent of total DM intake were 14.5, 14.6, 14.8, and 14.5% for the rations including concentrate mixtures containing corn, wheat, wheat plus protected amino acids and wheat with high RUP plus amino acids, respectively (Table III).

Digestibility of dry matter of the total ration was lower ($P<.05$) for cows fed the wheat ration supplemented with protected amino acids and calculated to be high in RUP than for cows fed the corn-base ration (Table III). This could be the result of the replacement of some soybean meal that is very digestible by less digestible feeds, corn gluten meal and blood meal. There was no difference in CP digestibility due to treatment.

Milk yield of cows fed the mixtures containing wheat calculated to be low in RUP with or without protected amino

TABLE III

FEED INTAKE BY COWS

Item	RATION			
	Control	Low (RUP)	Wheat (Low RUP+AA)	Wheat (High RUP+AA)
Dry matter intake kg/day				
Concentrate mix	14.83	14.85	14.69	14.84
Sorghum silage	12.05	12.01	11.83	11.91
Total	26.88	26.86	26.52	26.75
Protein intake				
Intake, kg/day	3.90	3.92	3.93	3.87
Requirement, kg/day	3.07	2.99	2.98	3.10
% of NRC reqmt	127	131	132	125
% of total DM intake	14.5	14.6	14.8	14.5
Aparent digestibility %[a]				
Dry matter	70.3[b]	66.4[bc]	66.3[bc]	62.8[c]
Crude protein	67.9	63.5	64.7	60.2

aDigestibility includes sorghum silage

bcMeans with different superscripts differ (P<.05).

acids was lower ($P<.01$) than that of cows fed the corn-based mixture and of cows fed the wheat mixture supplemented with protected amino acids and calculated to be high in RUP (Table IV). These responses in milk production could be a reflection of the amount of feed protein that escaped ruminal degradation and was available for digestion in the small intestine. Supplementation of the wheat mixture having low RUP with protected amino acids (lysine and methionine) alone had no effect on milk yield suggesting that amino acids other than lysine and methionine limited production. Whether or not replacement of soybean meal with corn gluten meal and blood meal would increase milk yield if the protected amino acids had not been added cannot be determined from data in this trial.

Milk fat test was slightly greater for the cows fed the wheat rations than for cows fed the corn-based ration (Table IV). This probably was merely a consequence of lower milk yield by cows fed the rations containing wheat. The average of 4% fat-corrected milk (29.2 kg/day) for the cows fed the wheat mixture supplemented with protected amino acids and high in RUP was significantly higher ($P<.05$) than that of cows fed the other wheat mixtures (28 kg/day), but not significantly different from that of those fed the corn-based mixture (28.9 kg/day).

Protein content of the milk of cows fed the protected amino acids was slightly higher (3.33 vs 3.25%) than that of cows not fed this supplement. Similarly, Schwab et al.

(1976) observed an increase in milk protein when cows were infused with both lysine and methionine, as did Donkin et al. (1987) who fed cows a corn based diet supplemented with rumen protected methionine and lysine. The slight increase may be a response to the increased supply of these two limiting amino acids at the site of milk protein synthesis.

The cows on the wheat mixtures had the tendency to gain more weight than cows on the corn-based mixture (Table V). Even though some of the differences were large, they were not statistically different. These results are in agreement with the results of Tommervik and Waldern (1969) where body weight gains were higher for cows fed wheat than for those fed corn rations, and in disagreement with the results by Faldet (1986) where the cows fed mixtures containing 60% wheat lost weight. There was no significant difference ($P>.05$) among treatments in changes in body condition score.

The ruminal pH at 2 hours after feeding was higher ($P<.02$) for cows fed the corn-based ration than for those fed the wheat rations (Table VI). Even though the pH in ruminal fluid was lower for cows fed the wheat mixtures, averaging 6.25, was considered to be normal for cows fed high concentrate rations. The ruminal pH values at 4 hours after feeding were similar for all treatments.

Ammonia-N concentration in the rumen fluid of the cows fed the wheat mixture high in RUP was lower ($P<.05$) than for the other treatments at 2 and 4 hours after feeding (Table

TABLE IV

MILK PRODUCTION AND COMPOSITION

Item	Control	Low (RUP)	Wheat (Low RUP+AA)	Wheat (High RUP+AA)
	RATION			
Milk Yield				
Milk, kg/day	30.8[a]	29.0[b]	29.1[b]	30.2[a]
Fat test, %	3.61	3.79	3.76	3.78
FCM, kg/day	28.9[cd]	28.0[c]	28.0[c]	29.2[d]
Protein, %	3.25	3.24	3.31	3.34
Protein, kg/day	1.00	.94	.96	1.01

[ab]Means with different superscripts differ ($P<.005$).

[cd]Means with different supercripts differ ($P<.04$).

TABLE V

BODY WEIGHT AND CONDITION SCORE CHANGE

Item	Control	Low (RUP)	Wheat (Low RUP+AA)	Wheat (High RUP+AA)
	RATION			
Weight change, kg/per	6.6	12.3	12.5	24.0
Condition score change per period	.2	.1	-.1	.2

TABLE VI

RUMEN PH, RUMEN AMMONIA AND BLOOD UREA

Item	RATION			
	Control	Low (RUP)	Wheat (Low RUP+AA)	Wheat (High RUP+AA)
Rumen pH, 2 hrs.	6.5[a]	6.2[b]	6.3[b]	6.2[b]
4 hrs.	6.3	6.3	6.2	6.3
Rumen ammonia-N, mg/dl				
2 hrs.	12.8[d]	13.2[d]	13.0[d]	9.5[e]
4 hrs.	8.8[d]	10.1[d]	9.9[d]	5.9[e]
Blood plasma urea-N mg/dl				
2 hrs.	20.8[fh]	23.7[g]	23.3[gh]	20.0[f]
4 hrs.	20.5	19.6	20.7	17.9

[ab]Means with different superscripts differ (P<.02).

[de]Means with different superscripts differ (P<.03).

[fgh]Means with different superscripts differ (P<.05).

VI). This could be a reflection of the lower degradability of some of the ingredients used in this mixture.

Blood plasma urea-N concentration at 2 hours after feeding was lower ($P<.05$) for the cows on the wheat mixture supplemented with amino acids and high in RUP than for cows fed the other wheat mixtures. Also, the blood plasma urea-N concentration for cows on the corn mixture was lower ($P<.05$) than for cows on the conventional wheat-based mixture (Table VI). However, the concentrations were relatively high in all groups, probably because protein intake exceeded requirements. At 4 hours after feeding, the concentration of blood urea-nitrogen was similar across treatments (Table VI).

The molar percentages of acetic acid at 2 and 4 hours after feeding were higher ($P<.05$) for cows fed the corn-base mixture than for cows on the unsupplemented wheat-base treatment (Table VII). Even though the differences in molar percentage of acetic acid were statistically different, they were small and consistent with normal milk fat test. Valeric acid was lower ($P<.01$) for cows on the corn-base mixture than for cows fed the two rations supplemented with the protected amino acids. Cows fed the unsupplemented wheat mixture had a lower molar percentage of valeric acid and were lower ($P<.05$) than the cows on wheat supplemented with amino acids at 2 hours after feeding. At 4 hours, the cows on the control corn-base mixture had a lower molar

TABLE VII

VFA CONCENTRATION IN RUMINAL FLUID

Item	RATION			
	Control	Wheat Low (RUP)	Wheat (Low RUP+AA)	Wheat (High RUP+AA)
Ruminal VFA (2 hr), molar %				
Acetic	62.5^{a}	59.8^{b}	60.9^{ab}	60.4^{ab}
Propionic	22.3	24.2	22.9	23.0
Isobutyric	.6	.5	.5	.5
Butyric	11.9	12.2	11.9	11.9
Isovaleric	1.3	1.7	2.0	2.3
Valeric	1.4^{a}	1.6^{ac}	1.8^{bc}	1.9^{b}
Total VFA concentration, mM/l	122.8	138.8	142.0	145.6
Acetic to propionic ratio	2.80	2.47	2.66	2.62
Ruminal VFA (4 hr), molar %				
Acetic	62.9^{a}	59.5^{b}	61.6^{ab}	61.8^{ab}
Propionic	21.9	24.2	22.5	22.4
Isobutyric	.6	.5	.6	.4
Butyric	11.6	12.0	11.7	12.0
Isovaleric	1.7	1.8	1.8	1.6
Valeric	1.3^{a}	1.9^{b}	1.8^{b}	1.7^{b}
Total VFA concentration, mM/l	124.0	144.7	135.6	128.6
Acetic to propionic ratio	2.87	2.46	2.74	2.76

^{abc}Means with different superscripts differ (P<.03).

percentage of valeric acid ($P<.03$) than cows fed the wheat mixtures.

Concentration of methionine in blood plasma was lower ($P<.05$) for the cows on the unsupplemented wheat mixture than for all other treatments. The concentration of lysine was lower ($P<.01$) for cows on the unsupplemented wheat mixture than for cows fed the supplemented wheat mixture low in RUP. There also were differences between groups of cows fed the two rations supplemented with protected amino acids, being lower ($P<.05$) for cows fed ration calculated to be high in RUP than for cows on the ration calculated to be low in RUP (Table VIII). These differences could be a result of the fact that the calculated amount of lysine did not agree with results obtained from lab analysis (Table IX). The wheat mixture calculated to be low in RUP and supplemented with amino acids had 1.24% lysine and the wheat mixture high in RUP supplemented with amino acids had .88% lysine. Plasma lysine and methionine concentrations were higher in cows fed the diets supplemented with amino acids than in cows fed the unsupplemented wheat ration. This was interpreted as evidence that these two amino acids were not limiting milk production for these cows.

TABLE VIII

CONCENTRATION OF AMINO ACIDS IN BLOOD PLASMA

Amino Acid	Control	Wheat Low (RUP)	Wheat (Low RUP+AA)	Wheat (High RUP+AA)
	RATION			
	(µM/100 ml)			
Lysine	9.1c	7.6d	10.4c	8.5d
Methionine	2.2a	1.7b	2.5a	2.4a
Threonine	9.9a	8.0b	9.6b	7.6a
Valine	26.7	26.2	27.7	27.9
Isoleucine	12.5a	11.8a	13.2a	9.6b
Leucine	17.7a	12.7b	12.9b	20.3a
Phenylalanine	5.0c	4.1d	4.4cd	5.0cd
Histidine	5.3	4.9	5.1	5.6
Arginine	8.1	7.3	9.8	7.1

[ab]Means with different superscripts differ ($P<.03$).

[cd]Means with different superscripts differ ($P<.05$).

TABLE IX

AMINO ACID CONTENT OF RATIONS[a]

Amino Acid	Control	Wheat Low (RUP)	Wheat (Low RUP+AA)	Wheat (High RUP+AA)
	RATION			
	(%,dry basis)			
Lysine	1.03	1.02	1.23	.88
Methionine	.28	.44	.36	.48
Threonine	.71	.71	.79	.70
Valine	.91	.92	1.08	1.05
Isoleucine	.79	.84	.92	.78
Leucine	1.64	1.51	1.71	2.11
Phenylalanine	.99	.99	1.14	1.10
Histidine	.46	.53	.58	.55
Arginine	1.38	1.42	1.56	1.12

[a]Results obtained from lab analysis

CHAPTER IV

SUBSTITUTING WHEAT FOR CORN IN CONCENTRATE MIXTURES FOR DAIRY COWS ON A WEIGHT BASIS

Summary

The effect of substituting wheat for corn on a weight rather than a protein basis was determined using 18 Holstein cows in their second or greater lactation. Treatments consisted of concentrate mixtures: (a) Control (75% corn, 12.1% protein), (b) Wheat (75% wheat, 15.1% protein), and (c) Wheat (75% wheat, 12.1% protein). The three rations were calculated to be isocaloric. Alfalfa hay was the only forage and constituted 50% of the ration on an air dry basis. Intake of dry matter was lower for cows fed the wheat rations than for those fed the control ration. Milk yield by cows fed the wheat rations was decreased with the yield of cows fed the ration where wheat was substituted for corn on a weight basis being intermediate to those of cows fed the other rations (33.7 vs. 34.5 and 33.0 kg/day). Milk fat and milk protein were similar for all treatments. Amount of protein in the rations did not affect body weight, rumen pH and molar percentages of acetic, propionic and butyric acids. However, it did affect body condition score

changes ($P<.05$) being highest for cows fed the control ration.

Introduction

A decline in the price of wheat has made it an attractive feed grain for livestock rations. As these prices stay lower than that of other cereal grains it becomes competitive and economically feasible to use more wheat in dairy rations.

Faldet et al. (1986 b) fed concentrate mixtures containing 0, 40, 60 and 80% wheat with alfalfa hay as the only forage. Milk yield declined as the amount of wheat increased (30.4, 29.8, 29.6 and 29.0 kg/day respectively). In that trial, the protein percentages of the concentrate mixtures were similar, averaging 12.2%. Waldern and Cedeno (1970) reported no differences in milk yield or fat percentage among group of cows fed concentrates of 98% barley or wheat mixed feed with alfalfa hay as the only rougage. Cunningham et al. (1970) fed lactating cows concentrate mixtures containing corn and either 33.3 or 66.7% of two varieties of soft red winter wheat (high protein, 18% and normal protein, 14%). A combination of alfalfa hay and corn silage (about 1:2.8 on dry matter basis) comprised the forage component of the ration. For cows fed either the low or high protein variety of wheat, milk yield and milk fat content were lower ($P<.05$) when wheat comprised 66.7 compared to 33.3% of the concentrate mix. However, average yield and composition of milk by cows fed the two wheat rations were similar to that of cows fed a control corn-based concentrate mixture. In that study, the protein percentages of the concentrate mixtures were similar across treatments, i.e., 15.3%.

The objective of this experiment was to determine the effect on the performance of dairy cows of substituting wheat for corn on a weight basis without adjusting for protein, using alfalfa hay as the only forage.

Materials and Methods

Eighteen Holstein cows in their second or greater lactation were used in a feeding trial starting 6 to 10 weeks postpartum. A switchback design with three 4-week periods was used (Lucas, 1956). The first two weeks were for adaptation to the rations and the last two weeks of each period were used for comparison among treatments. The cows were randomly assigned to treatment sequences. There were six treatment sequences, each of which included two treatments (Appendix, Table XVII). One treatment was given in the first and third periods and the second treatment during the second period.

The experimental rations were: (a) Control (75% corn base mixture, 12.1% protein), (b) Wheat (75% wheat, 15.1% protein), and, (c) Wheat (75% wheat, 12.1% protein). In the latter mixture most of the cottonseed meal was replaced with oats to get the level of protein equal to the control ration. The three rations were calculated to be nearly isocaloric (Table X). Alfalfa hay was the only forage and was 50% of the total ration. The cows were fed individually to appetite throughout the trial allowing for a minimum amount of weighback. The concentrate mixtures and the hay were each fed separately twice daily at 12-hour intervals. The alfalfa hay was fed 3 hours after the concentrate was fed each time.

Feed intake was recorded daily and alfalfa hay weighbacks were composited on a weekly basis for analysis of DM and CP. A sample of alfalfa hay and each of the concentrate mixtures also were analyzed for DM and CP each week for intake calculations. The analysis of dry matter was obtained by drying in a forced-air oven at 60 C for 48 hours and crude protein (N x 6.25) by the macro-kjeldahl method (A.O.A.C., 1975). Each cow was weighed on two consecutive days prior to the trial and during the last week of each period. The cows were weighed before milking time; therefore, the following milk weight was deducted. Also, body condition was evaluated prior to the trial and on the last day of each period using the scoring system described by Aalseth et al. (1983).

Cows were milked twice a day at 0500 and 1700 hours; milk weights were recorded daily and samples were collected at four consecutive milkings each week for determination of concentration of fat using a Milko Tester MK III F-3140 and

TABLE X

COMPOSITION OF CONCENTRATE MIXTURES FED WITH ALFALFA HAY[1]

Composition	Corn-base	Wheat-base (weight basis)	Wheat-base (protein basis)
	RATION		
Ingredients, (% as fed)			
Wheat[2]	--	75	75
Corn	75	--	--
Oats	4	4	14
Cottonseed meal	12	12	2
Cottonseed hulls	5	5	5
Dicalcium phosphate	1.75	1.75	1.75
Salt	.75	.75	.75
Sodium bicarbonate	1.00	1.00	1.00
Magnesium oxide	.50	.50	.50
Calculated analysis(as fed)			
Net energy Mcal/100 kg	164.3	164.3	163.7
Total protein, %	12.1	15.1	12.1
Rumen undeg. protein, %	6.2	3.8	2.6
Rumen undeg. lysine, %	.19	.12	.08
Rumen undeg. methionine, %	.11	.06	.04
Crude fiber, %	5.5	6.2	6.0

[1]Concentrate: hay, 50:50; calculated analysis of total ration (dry basis); NE_1 156.2 Mcal/100 kg.; crude fiber 18.8%; Ca .87%; P .51%.

[2]Hard red winter wheat, No.2 grade, test wt. 58 lb/bu.

protein concentration using a Pro-milk MK II F-12500 by the DHIA Lab.

During the last day of each period, 3 hours after the morning concentrate feeding, a sample of rumen fluid was collected by stomach tube. A minimum of 300 ml of fluid was collected and strained through a double layer of cheesecloth; pH was measured with a pH meter. Then, 200 ml of the strained fluid was mixed with 8 ml of 50% hydrochloric acid and frozen for later analysis. Later, the sample was thawed and analyzed for rumen ammonia nitrogen (NH3-N) following the procedure of Broderick and Kang (1980) using a Varian DMS 90 spectrophotometer at a wavelength of 630 nm. One hundred ml of the strained rumen fluid was mixed with 1 ml of saturated mercuric chloride and then frozen. Later, these samples were analyzed for VFA concentration. Samples were thawed and centrifuged (200 x g) for 10 minutes. Then, one ml of 20% meta-phosphoric acid was added to 5 ml of the supernatant solution and centrifuged for 20 minutes at (25,000 x g). The meta-phosphoric acid was used to convert the VFA that are in a salt form to a free acid form in order to determine VFA concentration by gas-liquid chromatography. One ml of supernatant solution was combined with .2 ml of 2-ethylbutyric acid (.5%) as an internal standard and samples were then subjected to gas-liquid chromatography for VFA analysis. At the same time the rumen samples were taken, a sample of blood from the median caudal vein was drawn into a

15 ml vacutainer tube and mixed with .15 ml of oxalic acid to prevent coagulation and immediately put into ice-water. Samples were centrifuged at 2000 x g for 30 minutes; plasma was separated and then frozen. Later, the samples were analyzed for plasma urea nitrogen concentration by the method described by Fawcett et al. (1960).

Data collected during the trial for the different response variables were analyzed by analysis of variance, using the Statistical Analysis System (SAS). Adjusted treatment means were compared using pre-planned orthogonal contrasts as follows: control corn-based mixture vs. wheat mixture with 15.1% protein and wheat mixture with 12.1% protein; wheat mixture with 15.1% protein vs. wheat mixture with 12.1% protein.

Results and Discussion

Intake of concentrate dry matter was lower for both wheat groups than for cows on the corn mixture (12.0 and 11.9 vs. 12.5 kg/day for the wheat with 15.1% protein, wheat with 12.1% protein and corn ration, respectively). Hay dry matter intake was lower ($P<.05$) for cows on the wheat mixture with 12.1% protein than for cows on the corn mixture (Table XI). The decrease in feed intake was consistent with the results of Faldet et al. (1986b) in that they observed a decrease in intake as the amount of wheat in the concentrate mixture increased. The average ratio of the concentrate and roughage consumed was 51:49.

TABLE XI

FEED INTAKE BY COWS

Item	Ration/percent protein		
	Corn 12.1%	Wheat 15.1%	Wheat 12.1%
DM Intake, kg/day			
Concentrate mix	12.5^{a}	12.0^{b}	11.9^{b}
Hay	11.8^{a}	11.6^{ab}	11.2^{b}
Total	24.3^{a}	23.5^{b}	23.1^{b}
Protein intake, kg/day			
Concentrate mix	1.72^{d}	2.07^{e}	1.64^{d}
Hay	2.41^{a}	2.38^{a}	2.31^{b}
Total	4.13^{a}	4.45^{b}	3.95^{c}
Prot. requirement, kg/day	3.23	3.16	3.13
Prot. % of NRC reqmt.	128	141	126
Prot. % of total DM intake	17.0	18.9	17.1

[abc]Means with different superscripts differ ($P<.05$).

[de]Means with different superscripts differ ($P<.0001$).

Intake of total crude protein was significantly higher ($P<.01$) for the cows on the wheat mixture with 15.1% protein than for the other groups, as expected. Also, intake of protein by cows in the control group was higher ($P<.05$) than that of cows fed the wheat ration containing 12.1% protein (Table XI) due to greater intake of dry matter. However, intake of protein by all groups exceeded NRC requirements for total protein. The excess was 27% for the rations with 12.1% protein and 41% for cows on the 15.1% protein. This occurred because expected production was higher than actually was produced, and quality of alfalfa hay was higher than expected.

Milk yield for the cows fed the wheat mixture containing the 12.1% protein was lower ($P<.06$) than that of cows fed the control ration (33.0 vs. 34.5 kg/day) (Table XII). This decline was greater than that observed by Faldet et al. (1986b) where wheat comprised 60% of the concentrate mixture, possibly because in this trial cows produced more milk and were more responsive to differences in ration content. The response of substituting wheat for corn on milk production was less severe when the amount of protein derived from other components of the ration was not reduced. This was consistent with results of the first trial where SBM was replaced by less degradable ingredients to maintain a similar undegradable protein level to allow more protein to be absorbed in the small intestine.

TABLE XII

MILK PRODUCTION AND COMPOSITION

	Ration/percent protein		
Item	Corn 12.1%	Wheat 15.1%	Wheat 12.1%
Milk yield			
Milk, kg/day	34.5^{a}	33.7^{ab}	33.0^{b}
Fat test, %	3.18	3.14	3.22
FCM, kg/day	30.3	29.3	29.0
Protein, %	2.87	2.82	2.86
Protein, kg/day	.99	.95	.94

^{ab}Means with different superscripts differ (P<.06)

The percentage of milk fat and protein were similar across treatments (Table XII) although lower than expected, since alfalfa hay constituted 48.8% of the total dry matter intake by the cows and had an acid-detergent fiber content of 34.1% (dry basis). Mineral buffers, sodium bicarbonate and magnesium oxide, were also added to all the concentrate mixtures for prevention of acidosis and milk fat depression.

There were no significant differences in body weight ($P>.05$) observed in the cows with different amounts of protein intake. However, the condition score change for cows fed the corn mixture was significantly greater ($P<.05$) than that of cows fed the wheat mixtures (0.42 vs .03 and -.04) (Table XIII). These increases in body condition may be critical for the next lactation if maximum milk production is expected.

Rumen pH was not affected by the amount of protein in the diets (Table XIV). The values were above those generally observed to be associated with digestive disorders.

Rumen ammonia-N levels were higher ($P<.05$) for cows on the wheat mixture with 15.1% protein (9.7 mg/dl) than for cows on the corn-base mixture or wheat with 12.1% protein (7.0 and 7.1 mg/dl, respectively) (Table XIV). This difference was expected because of the greater protein intake by the cows on that ration. Similarly, Kung and Huber (1983) observed that rumen ammonia nitrogen tended to increase with increased ration protein. Blood urea-N levels

TABLE XIII

BODY WEIGHT AND CONDITION SCORE CHANGE

Item	Ration/percent protein		
	Corn 12.1%	Wheat 15.1%	Wheat 12.1%
Weight change, kg/period	8.41	9.20	9.94
Condition score change/per	0.42[a]	0.03[b]	-0.04[b]

[ab]Means with different superscripts differ ($P<.05$).

TABLE XIV

RUMEN PH, RUMEN AMMONIA AND BLOOD UREA

Item	Ration/percent protein		
	Corn 12.1%	Wheat 15.1%	Wheat 12.1%
Ruminal fluid pH	6.3	6.2	6.2
Rumen ammonia-N mg/dl	7.0[a]	9.7[b]	7.1[a]
Blood plasma urea-N mg/dl	20.1	22.7	19.2

[ab]Means with different superscripts differ ($P<.05$)

were similar across treatments (Table XIV). The high concentrations were expected because the protein intakes were greater than NCR recommendations and similar to those observed in a previous trial.

Molar proportions of acetic, propionic, butyric and valeric acid did not differ among levels of protein and were similar across treatments (Table XV). The ratio of acetic to propionic acid averaged 2.8:1 and was consistent with normal milk fat test. Molar percentage of isobutyric and isovaleric were lower ($P<.05$) for the cows on the wheat ration with 15.1% protein than that of cows on the corn base mixture. Replacement of corn by wheat on a weight basis could be an alternative to feed lactating dairy cows without loss of production.

TABLE XV

VFA CONCENTRATION IN RUMINAL FLUID

Item	Ration/percent protein		
	Corn 12.1%	Wheat 15.1%	Wheat 12.1%
Acid, molar %			
Acetic	62.9	62.3	61.9
Propionic	21.9	22.5	22.9
Butyric	11.3	11.9	11.2
Isobutyric	.7[a]	.5[b]	.5[b]
Valeric	1.9	1.9	2.1
Isovaleric	1.4[c]	.9[d]	1.2[cd]
Acetic to propionic ratio	2.9	2.8	2.7

[ab]Means with different superscripts differ (P<.06).

[cd]Means with different superscripts differ (P<.03).

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

The incorporation of hard red winter wheat in dairy rations without limiting production can be achieved. However, in some studies, milk yield has been reduced with the incorporation of large amount of wheat in dairy mixtures. The amount of rumen undegradable protein (RUP) was reduced; in particular, the amount of lysine and methionine that would be expected to escape ruminal degradation was lowered. Two trials were conducted to determine whether or not altered protein or amino acid supplies is responsible for a decline in milk yield when cows are fed rations containing large amounts of wheat. In the first trial, cows were fed rations with sorghum silage as the only roughage in a 55:45 ration. The concentrate mixtures were: (a) positive control (with corn at 45 and barley at 10% of the concentrate mix), (b) negative control (wheat at 60%), (c) diet "b" plus supplemental protected lysine and methionine, and (d) high wheat mixture with protected amino acids plus RUP equal to the positive control. Dry matter intake was similar for all treatment groups, with average intake of wheat of 8.9 kg/day for cows on the wheat rations. Milk yield of cows fed the mixtures containing wheat calculated to be low in RUP, with or without protected amino acids, was lower than for cows fed the positive control ration. However, milk production was similar for cows fed the wheat ration high in RUP and those fed the control ration. Milk fat and protein were not affected by treatments.

In the second trial, cows were fed concentrate mixtures and alfalfa hay as the only roughage in a 50:50 ratio. The experimental rations were: (a) control (75% corn-base mixture, 12.1% protein), (b) wheat (75% wheat base, 15.1% protein), and (c) wheat (75% wheat base, 12.1% protein). All rations were calculated to be isocaloric. Intake of dry matter from both concentrate and hay were affected with intake being lower for cows fed either of the wheat rations than for cows fed the corn base ration. Milk yield for cows fed the wheat mixture containing the 12.1% protein was lower than that of cows fed the control ration, but milk yield for cows on the wheat ration with 15.1% protein was intermediate. Milk fat and protein were similar across treatments.

In both trials, responses in milk yield was achieved when the ammount of rumen undegradable protein was increased either by using less degradable ingredients or by an increase in the increment of total protein when wheat substituted for corn on a weight basis. Supplementation of a wheat mixture with protected amino acids did not affect milk production.

Wheat could be an alternative feed source for lactating dairy cows if the price of wheat were low enough to make it competitive with corn. The amount of rumen undegradable protein has to be taken into consideration when formulating rations wherein wheat is included in large amounts, because wheat is highly degraded in the rumen and an increase of undegraded protein to the intestine is necessary in order to make wheat competitive to corn for milk production.

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APPENDIX

TABLE XVI

TREATMENT SEQUENCE CODES - TRIAL 1

Treatment Sequence[a]	Code Number
1-2-1	1
2-3-2	2
3-4-3	3
4-1-4	4
1-3-1	5
2-4-2	6
3-1-3	7
4-2-4	8
1-4-1	9
2-1-2	10
3-2-3	11
4-3-4	12

[a]Control= 1; Wheat (Low RUP)= 2; Wheat (High RUP+AA)= 3; Wheat (Low RUP+AA)= 4.

TABLE XVII

TREATMENT SEQUENCE CODES - TRIAL 2

Treatment Sequence[a]	Code Number
1-2-1	1
2-3-2	2
3-1-3	3
1-3-1	4
2-1-2	5
3-2-3	6

[a]Corn 12.1% CP= 1; Wheat 15.1% CP=2; Wheat 12.1% CP= 3

TABLE XVIII

LACTATION, CALVING DATE, TREATMENT SEQUENCE, POSTPARTUM DAYS WHEN STARTED, AND MILK PRODUCTION PER PERIOD FOR EACH COW TRIAL 1

Cow No.	Lact. No.	Calving date, 85	Trt. Seq.	Post-partum days	Avg. milk yield (kg/day) Per. 1	Per. 2	Per. 3
294	4	8/28	2	58	32.9	31.2	28.1
331	3	8/29	3	57	35.5	27.2	34.9
534	2	8/31	4	55	26.9	25.3	20.9
521	2	9/1	5	54	32.1	32.0	30.5
340	4	9/1	6	54	29.1	25.8	22.9
455	3	9/2	7	53	32.1	29.8	28.3
376	3	9/2	8	53	30.5	26.4	26.6
419	3	9/3	9	59	32.2	29.4	29.2
404	3	9/3	10	59	27.6	26.8	24.9
529	2	9/5	12	57	34.2	32.0	31.4
076	6	9/5	12	57	31.0	28.5	28.5
008	6	9/7	1	55	32.5	31.7	32.7
061	6	9/10	3	52	31.9	27.2	26.6
338	4	9/10	4	52	37.4	36.6	33.1
327	3	9/11	5	58	42.2	38.3	35.0
456	3	9/11	6	58	31.1	27.6	27.1
303	4	9/12	7	59	34.8	31.6	31.5
067	6	9/18	8	51	28.7	20.7	26.6
545	2	10/8	9	51	31.1	28.1	28.7
316	4	10/9	10	50	34.2	35.4	32.5
514	2	10/10	11	49	35.2	31.6	32.3
039	6	10/15	12	44	31.5	28.3	26.1
524	2	9/26	1	64	25.4	22.1	23.4
059	6	9/29	2	61	31.4	30.8	27.0

TABLE XIX

LACTATION, CALVING DATE, TREATMENT SEQUENCE, POSTPARTUM DAYS WHEN STARTED, AND MILK PRODUCTION PER PERIOD FOR EACH COW TRIAL 2

Cow No.	Lact. No.	Calving date, 85	Trt. Seq.	Postpartum days	Avg. milk yield (kg/day) Per. 1	Per. 2	Per. 3
289	3	12/4	1	72	36.8	40.2	34.8
329	4	12/6	2	70	31.0	30.1	29.3
149	6	12/18	4	58	37.7	36.4	32.0
489	2	12/19	5	57	32.2	31.7	31.0
380	3	12/25	3	58	40.8	36.4	32.3
121	6	12/28	2	55	42.2	38.8	33.8
402	3	1/4	6	51	29.3	27.8	24.0
378	3	1/11	3	55	36.3	36.9	34.2
215	4	1/13	5	53	42.1	41.7	36.6
382	3	1/16	6	50	41.3	38.9	35.1
325	3	1/20	4	46	36.9	33.4	34.3
311	4	1/22	2	44	34.0	32.4	28.1
532	2	1/23	3	43	30.8	31.2	26.9
531	2	1/29	1	44	34.9	29.6	27.0
241	3	1/29	4	44	38.0	35.6	33.0
160	5	2/5	5	44	38.0	37.6	34.9
390	3	2/14	6	42	35.5	34.5	28.9

TABLE XX

ANALYSIS OF WHEAT, % DRY BASIS

Item	Wheat 1[a]	Wheat 2[a]	Sorghum[b] Silage	Alfalfa[b] Hay
Dry matter	90.8	91.0	38.5	87.8
Crude protein	14.8	16.7	6.7	20.2

[a]Hard red winter wheat grain analysis in Trial 1 and 2 (1 observation)

[b]Weekly samples (4 samples per period)

TABLE XXI

USDA GRADE ANALYSIS[a]

	US grade no.				Wheat[b]	
Item	1	2	3	4	Exp. 1	Exp. 2
Min. test weight per bushel, lb	60	58	56	54	60	58
Damaged kernels (total), %	2	4	7	10	2.5	1.8
Foreign material, %	.5	1	2	3	.7	2

[a]US Standards for Grains, USDA, Revised Dec. 1975.

[b]One observation per experiment.

TABLE XXII

ANALYSIS OF VARIANCE FOR MILK YIELD - TRIAL 1

Source[a]	Degrees of freedom	Sum of squares	F Value	P>F[b]
Cow	23	4652.97	57.07	.0001
Per (Cow)	24	743.63	8.74	.0001
Per x Per	1	6.27	1.77	.1985
Treatment	3	119.94	11.28	.0002
Error	20	70.90		
Corrected total	71	5593.72		

[a]Abbreviation for period (Per)

[b]Probability of a larger F value.

TABLE XXIII

ANALYSIS OF VARIANCE FOR MILK YIELD - TRIAL 2

Source[a]	Degrees of freedom	Sum of squares	F Value	P>F[b]
Cow	16	3014.62	12.40	.0001
Per (Cow)	17	1629.68	6.31	.0006
Per x Per	1	54.78	3.60	.0784
Treatment	2	67.85	2.23	.1441
Error	14	212.79		
Corrected total	50	4979.73		

[a]Abbreviation for period (Per)

[b]Probability of a larger F value.

TABLE XXIV

COEFFICIENTS OF VARIATION FOR RESPONSE VARIABLES

Response[a]	Trial 1	Trial 2
Con. DM intake	4.8	3.3
Roughage DM intake	5.4	4.7
Total DM intake	5.0	2.5
DM digestibility	8.6	--
Total CP intake	5.1	4.4
CP digestibility	9.7	--
Milk yield	2.9	5.3
Milk fat percent	5.5	12.2
4% FCM	4.1	7.5
Milk protein percent	3.4	3.9
Body weight change	170.6	278.0
Condition score change	470.3	267.0
Rumen pH (2 hrs.)	2.5	3.6
(4 hrs.)	5.4	--
Rumen ammonia-N (2 hrs.)	21.1	35.5
(4 hrs.)	31.2	--
Blood plasma urea-N (2 hrs.)	14.5	21.4
(4 hrs.)	17.3	--

[a]Abbreviation for sources are 2 hours after feeding (2 hrs.) and 4 hours after feeding (4 hrs.)

TABLE XXIV (Continued)

Response[a]	Trial 1	Trial 2
VFA		
Acetic (2 hrs.)	4.3	5.6
(4 hrs.)	4.9	--
Propionic (2 hrs.)	11.5	18.9
(4 hrs.)	15.2	--
Butyric (2 hrs.)	13.7	22.0
(4 hrs.)	13.7	--
Isobutyric (2 hrs.)	28.3	36.1
(4 hrs.)	28.1	--
Valeric (2 hrs.)	13.6	27.8
(4 hrs.)	14.8	--
Isovaleric (2 hrs.)	80.0	36.2
(4 hrs.)	25.7	--
Blood amino acids		
Lysine	17.3	--
Methionine	21.1	--
Threonine	17.8	--
Valine	14.0	--
Isoleucine	17.0	--
Leucine	20.3	--
Phenylalanine	16.4	--
Histodine	9.1	--
Argenine	17.4	--

[a]Abbreviation for sources are 2 hours after feeding (2 hrs.) and 4 hours after feeding (4 hrs.)

TABLE XXV

Cr_2O_3 DETERMINATION PROCEDURE

Ash 0.4 g of sample

Transfer ashed sample into 100 ml Erlemmeyer flask

Add 6 ml of acid mixture[a]

Bring to boil

Add 3 ml of 4.5% $KBrO_3$ and continue boiling until one minute after SO_3 fumes appear

Remove from heat and cool for 10 minutes

Bring up to 100 ml

Transfer 5 ml to centrifuge tube

Add 7.5 ml of 5% NaOH

Vortex no less than 15 minutes

Allow to settle at least 45 minutes

Centrifuge for 15 minutes at 2000 rpm

Read at 400 nm

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[a]Acid mixture

500 ml distilled water
250 ml H_2SO_4
250 ml H_3PO_4
50 ml 10% solution $MNSO_4$-$4H_2O$

VITA

Tor Nalsen

Candidate for the Degree of

Master of Science

Thesis: EFFECT OF AMINO ACID SUPPLEMENTATION AND SOURCE OF PROTEIN IN HIGH-WHEAT MIXTURES ON RESPONSES OF LACTATING DAIRY COWS.

Major Field: Dairy Science

Biographical:

Personal Data: Born in Caracas, Venezuela, September 23, 1954, the son of Svante Nalsen and Margarita Ostlund de Nalsen, married to Sherry Woods Nalsen. Children: Tomese Michelle and Thor Daniel.

Education: Graduated from Instituto Victegui High School, Los Teques, Venezuela, July, 1972; Received a Bachelor of Science in agriculture at Oklahoma State University, 1980; completed requirements for Master of Science Degree in Dairy Science at Oklahoma State University, December, 1987.

Experience: Internship at Fort Reno Research Station, El Reno, Oklahoma, summer 1980. Manager of Hacienda Catire Antonio, Mene Grande, Venezuela, 1981-1983. Dairy Technician, Oklahoma State University Dairy Cattle Center, summer 1983. Research technician, Oklahoma State University Dairy Cattle Center, September, 1983 to December, 1987.

Membership: American Dairy Science Association.